

CLAIMS:

5
Sub
C2

10
1. A method for detecting the presence or absence of twelve mutations in the cystic fibrosis transmembrane conductor regulator (CFTR) gene, which method comprises contacting sample genomic DNA from an individual in two separate reaction vessels with allele specific primer sets for (A) 1717-1 G>A, G542X, W1282X, N1303K, ΔF508(M), 3849+10kb C>T mutations and (B) the 621+1 G>T, R553X, G551D, R117H, R1162X and R334W mutations respectively, in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that each diagnostic primer is extended only when the relevant mutation is present in the sample; and detecting the presence or absence of CFTR gene alleles by reference to the presence or absence of diagnostic primer extension product(s).

15
2. A method as claimed in claim 1 and wherein one or more diagnostic primers is used with one or more amplification primers in one or more cycles of PCR amplification.

20
3. A set of allele specific primers for each of the following alleles of the CFTR gene: 1717-1 G>A, G542X, W1282X, N1303K, ΔF508(M), and 3849+10kb C>T mutations.

4. A set of primers as claimed in claim 3 and comprising the following diagnostic primer sequences:

TCTTGGGATTCAATAAATTTCGAACAGTCA
TACTAAAAGTGACTCTCTAATTTTCTATTTTGGTAATTA
AGTTTGCAAGAGAAAGACAATATAGTTCTCT
TGATCACTCCACTGTTTCATAGGGATCCATC
GTATCTATATTCATCATAGGAAACACCAT
ACATTTCTTTTCAGGGTGTCTGACTAA

25
5. A set of allele specific primers for each of the following alleles of the CFTR gene: 621+1 G>T, R553X, G551D, R117H, R1162X and R334W mutations.

6. A set of primers as claimed in claim 5 and comprising the following diagnostic primer sequences:

GTATCTATATTCATCATAGGAAACACCACA
TGCCATGGGGCCTGTGCAAGGAAGTATTGA
AGCCTATGCCTAGATAAATCGCGATAGACT
CCTATGCACTAATCAAGGAATCATCCTGT
GCTAAAGAAATTCCTGCTCGTTGTT
GACTGACTGACTGACTGACTCTGACTGACTTATTCA
CCTTGCTAAAGAAATTCCTGCTGA
TATTTTATTTTCAAGATGCGATCTGTGAGTT

7. A set of primers comprising the following diagnostic primer and amplification primer sequences:

TCTTGGGATTCAATAACTTTGCAACAGTCA
GAATCCCAAACCTTTAGAGACATC
TACTAAAAGTGACTCTCTAATTTTCTATTTTGGTAATTA
AGTTTGAGAGAAAGACAATATAGTTCTCT
TAATCTCTACCAAATCTGGATACTATACC
TGATCACTCCACTGTTTCATAGGGATCCATC
AATTTGAGAGAACTTGATGGTAAGTACA
GTATCTATATTCATCATAGGAAACACCATT
CCAGACTTCACCTCTAATGATGATTATGGG
ACATTTCTTTTCAAGGTGCTGACTAA
TTGTGGATCAAATTCAGTTGACTTGTCTATC

8. A set of primers comprising the following diagnostic primer and amplification primer sequences:

GTATCTATATTCATCATAGGAAACACCACA
GACTTCACCTCTAATGATGATTATGGGAGA
TGCCATGGGGCCTGTGCAAGGAAGTATTGA
AGCCTATGCCTAGATAAATCGCGATAGACT
GTTTCACATAGTGTATGACCCTCTATATACACTCATT
CCTATGCACTAATCAAAGGAATCATCCTGT
TTTGTTTATTGCTCCAAGAGAGTCATACCA
GCTAAAGAAATTCCTGCTCGTTGTT
GACTGACTGACTGACTGACTCTGACTGACTTATTCA

CCTTGCTAAAGAAATTCTTGCTGA
 TAAAATTGGAGCAATGTTGTTTTGACC
 TATTTTATTTTCAGATGCGATCTGTGAGTT
 TTTTGCTGTGAGATCTTTGACAGTCATT

9. A set of primers as claimed in any one of the previous claims and comprising one or more of the following control primers:

GAGCACAGTACGAAAAACCACT
 AAACTTTTACAGGGATGGAGAACG
 AGAGGATTATCTATGCAAATCCTTGTAACC
 TCAACTTCACTATCAAAAGTCATCATCTAG

10. A diagnostic kit for detecting the presence or absence of twelve mutations in the cystic fibrosis transmembrane conductor regulator (CFTR) gene which comprises sets of primers as claimed in any one of the previous claims.

Add
A

add B5

add C17

add D7

5
 66270-000000